

CLAIMS

What Is Claimed Is:

- 1 1. A method of detecting G protein-coupled receptor (GPCR) pathway activity,
2 comprising:
3 providing at least one cell that expresses a GPCR and a plurality of
4 conjugated proteins, each of the plurality of conjugated proteins formed by
5 conjugating an arrestin protein and a detectable molecule, the plurality of conjugated
6 proteins being substantially evenly distributed in the cytoplasm of the at least one
7 cell;
8 obtaining a first image of the at least one cell by detecting an amount of
9 energy emitted from the detectable molecules and storing a value relative to the
10 amount of energy;
11 treating the at least one cell with a test compound;
12 obtaining a second image of the at least one cell by detecting an amount of
13 energy emitted from the detectable molecules and storing a value relative to the
14 amount of energy; and
15 comparing the first image and the second image to detect the localization of
16 at least some of the plurality of conjugated proteins at at least one of endocytic
17 vesicles and endosomes.
- 1 2. The method of claim 1, wherein the step of obtaining a second image of the
2 at least one cell includes setting a threshold intensity such that the energy emitted
3 from detectable molecules evenly distributed in the cytoplasm are excluded.
- 1 3. The method of claim 2, wherein the threshold intensity is set to exclude as
2 much as possible of the energy emitted from the detectable molecules that are
3 evenly distributed in the cytoplasm and as little as possible of the energy emitted
4 from the detectable molecules in endocytic vesicles.

1 4. The method of claim 2, wherein the threshold intensity is set at the mean
2 intensity of all energy emitted in a control cell plus two standard deviations.

1 5. The method of claim 2, wherein the threshold intensity is set at the mean
2 intensity of all energy emitted in a control cell plus three standard deviations.

1 6. The method of claim 1, wherein the test compound is a potential agonist or a
2 potential antagonist.

1 7. A method of detecting G protein-coupled receptor (GPCR) pathway activity,
2 comprising:

3 providing at least one cell that expresses a GPCR and a plurality of
4 conjugated proteins, each of the plurality of conjugated proteins formed by
5 conjugating an arrestin protein and a detectable molecule, the plurality of conjugated
6 proteins being substantially evenly distributed in the cytoplasm of the at least one
7 cell;

8 obtaining a first digital image of the at least one cell by detecting and
9 measuring energy emitted from the detectable molecules, the first digital image
10 being formed from an array of a plurality of pixels each having respective intensity
11 values, a respective intensity value being based on the intensity of energy emitted
12 from the detectable molecules associated with a pixel's location in the array;

13 treating the at least one cell with a test compound;

14 obtaining a second digital image of the at least one cell by detecting and
15 measuring energy emitted from the detectable molecules, the second digital image
16 being formed from an array of a plurality of pixels each having respective intensity
17 values, a respective intensity value being based on the intensity of energy emitted
18 from the detectable molecules associated with a pixel's location in the array; and

19 comparing the first digital image and the second digital image to detect the
20 localization of at least some of the plurality of conjugated proteins at at least one of
21 endocytic vesicles and endosomes, the localization of at least some of the plurality
22 of conjugated proteins being detected by a change in apparent intensity of energy

emitted from detectable molecules resulting in an increase in the value of at least some of the plurality of pixels.

8. A method of detecting G protein-coupled receptor (GPCR) pathway activity, comprising:

- providing at least one cell that expresses a GPCR and a plurality of conjugated proteins, each of the plurality of conjugated proteins formed by conjugating an arrestin protein and a detectable molecule, the plurality of conjugated proteins being substantially evenly distributed in the cytoplasm of the at least one cell;
- treating the at least one cell with a test compound;
- obtaining a digital image of the at least one cell by detecting and measuring energy emitted from the detectable molecules, the digital image being formed from an array of a plurality of pixels each having respective intensity values, a respective intensity value being based on the intensity of energy emitted from the detectable molecules associated with a pixel's location in the array; and
- detecting the localization of at least some of the plurality of conjugated proteins at at least one of endocytic vesicles and endosomes, the localization of at least some of the plurality of conjugated proteins being detected by a change in apparent intensity of energy emitted from detectable molecules resulting at least some of the plurality of pixels having a value above a threshold intensity.

9. The method of claim 8, wherein the step of obtaining a digital image of the at least one cell includes setting the threshold intensity such that the energy emitted from detectable molecules evenly distributed in the cytoplasm are excluded.

10. The method of claim 9, wherein the threshold intensity is set to exclude as much as possible of the energy emitted from the detectable molecules that are evenly distributed in the cytoplasm and as little as possible of the energy emitted from the detectable molecules in endocytic vesicles.

1 11. The method of claim 8, wherein the threshold intensity is set based on the
2 mean intensity of all energy emitted from detectable molecules in a control cell.

1 12. The method of claim 11, wherein the threshold intensity is set at the mean
2 intensity of all energy emitted from detectable molecules in a control cell plus two
3 standard deviations.

1 13. The method of claim 11, wherein the threshold intensity is set at the mean
2 intensity of all energy emitted from detectable molecules in a control cell plus three
3 standard deviations.

1 14. The method of claim 8, wherein the test compound is a potential agonist or a
2 potential antagonist.

1 15. A method of detecting G protein-coupled receptor (GPCR) pathway activity,
2 comprising:

3 providing at least one cell that expresses a GPCR and a plurality of
4 conjugated proteins, each of the plurality of conjugated proteins formed by
5 conjugating an arrestin protein and a detectable molecule, the plurality of conjugated
6 proteins being substantially evenly distributed in the cytoplasm of the at least one
7 cell;

8 treating the at least one cell with a test compound;

9 obtaining a digital image of the at least one cell by detecting and measuring
10 energy emitted from the detectable molecules, the digital image being formed from
11 an array of a plurality of pixels each having respective intensity values, a respective
12 intensity value being based on the intensity of energy emitted from the detectable
13 molecules associated with a pixel's location in the array; and

14 detecting the localization of at least some of the plurality of conjugated
15 proteins by a change in apparent intensity of energy emitted from detectable
16 molecules resulting in at least some of the plurality of pixels having a value above a
17 threshold intensity.

1 16. The method of claim 15, wherein the step of obtaining a digital image of the
2 at least one cell includes setting the threshold intensity such that the energy emitted
3 from detectable molecules evenly distributed in the cytoplasm are excluded.

1 17. The method of claim 16, wherein the threshold intensity is set to exclude as
2 much as possible of the energy emitted from the detectable molecules that are
3 evenly distributed in the cytoplasm and as little as possible of the energy emitted
4 from the detectable molecules in endocytic vesicles.

1 18. The method of claim 15, wherein the threshold intensity is set based on the
2 mean intensity of all energy emitted from detectable molecules in a control cell.

1 19. The method of claim 18, wherein the threshold intensity is set at the mean
2 intensity of all energy emitted from detectable molecules in a control cell plus two
3 standard deviations.

1 20. The method of claim 18, wherein the threshold intensity is set at the mean
2 intensity of all energy emitted from detectable molecules in a control cell plus three
3 standard deviations.

1 21. The method of claim 15, wherein the test compound is a potential agonist or
2 a potential antagonist.